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L1: Entry 1 of 1 File: USPT Sep 4, 2001

US-PAT-NO: 6284494
DOCUMENT-IDENTIFIER: US 6284494 B1

TITLE: Methods and compositions for synthesis of oligosaccharides using mutant glycosidase enzymes

DATE-ISSUED: September 4, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CAX
MacKenzie; Lloyd	Vancouver			CAX
Wang; Qingping	Kirkland			CAX

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The University of British Columbia	Vancouver			CAX	03

APPL-NO: 9/ 091272
DATE FILED: September 29, 1998

PARENT-CASE:
This application is a U.S. National Phase, filed under 35 USC .sctn. 371, of PCT/CA96/00841, which is a continuation-in-part of U.S. patent application Ser. No. 08/571,175 filed Dec. 12, 1995, now U.S. Pat. No. 5,716,812.

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/CA96/00841	December 12, 1996	WO97/21822	Jun 19, 1997	Sep 29, 1998	Sep 29, 1998

INT-CL: [7] C12P 19/44, C12P 19/12, C12N 9/24, C12N 9/26, C12N 9/42
US-CL-ISSUED: 435/74; 435/100, 435/200, 435/201, 435/209
US-CL-CURRENT: 435/74; 435/100, 435/200, 435/201, 435/209
FIELD-OF-SEARCH: 435/74, 435/100, 435/200, 435/201, 435/209, 435/440

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4918009	April 1990	Nilsson	435/73
☐	5246840	September 1993	Nilsson	435/101
☐	5372937	December 1994	Nilsson	435/74

FOREIGN PATENT DOCUMENTS

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US-PAT-NO: 6284494
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TITLE: Methods and compositions for synthesis of oligosaccharides using mutant glycosidase enzymes

DATE-ISSUED: September 4, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CAX
MacKenzie; Lloyd	Vancouver			CAX
Wang; Qingping	Kirkland			CAX

US-CL-CURRENT: 435/74; 435/100, 435/200, 435/201, 435/209

CLAIMS:

What is claimed is:
1. A method for synthesizing an oligosaccharide comprising the steps of:
(a) combining a glycosyl donor molecule and a glycoside acceptor molecule in a reaction mixture, said glycosyl donor molecule having a .beta. configuration and said glycoside acceptor molecule having an .alpha. configuration, or vice versa; and
(b) enzymatically coupling the donor molecule to the acceptor molecule using Agrobacterium .beta.-glucosidase in which amino acid 358 has been changed from glutamic acid to an amino acid with a non-carboxylic acid side chain.
2. The method of claim 1, wherein the, amino acid 358 has been changed from glutamic acid to alanine.

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0226563	June 1987	EPX	
87/05936	October 1987	WOX	
89/09275	October 1989	WOX	
94/29477	December 1994	WOX	
95/18864	July 1995	WOX	
95/18232	July 1995	WOX	

OTHER PUBLICATIONS

Withers et al., "Mechanistic Comsequences of Mutation of the Active Site Nucleophile GLU 358 in Agrobacterium .beta.-Glucosidase" Biochemistry 31: 9979-9985 (1992).
Trimbur et al., A .beta.-Glucosidase from an Agrobacterium sp.: Structure and Biochemistry in ACS Sympsium Series (1992) pp. 42-55.
Gebler et al., "Substrate-Induced Inactivation of a Crippled .beta.-Glucosidase Mutant: Identification of the labeled Amino Acid and Mutagenic Analysis of Its Role", Biochemistry 34: 14547-14553 (1995).
Wang et al., "Identification of the Acid/Base catalyst in Agrobacterium faecalis .beta.-glucosidase by kinetic analysis of mutants" Biochemistry 34: 14454-14562 (1995).
Wang et al., "Substrate-assisted Catalysis in Glycosidases" J. Amer. Chem. Soc. 117: 10137-1-138 (1995).
Witt et al., "6-Phospho-.beta.-galactosidases of Gram Positive and 6-phospho-.beta.-glucosidase B of Gram-Negative bacteria: comparison of structure and function by kinetic and immunological methods and mutageneisis of the lacG gene of Staphyloccous aureus" Protein Engineering 6: 913-920 (1993).
Nikolova et al., "Transglycosylation by Wild Type and Mutants of a .beta.-1,4-Glycosidase from Cellulomonas fimi (Cex) for synthesis of Oligosaccharides", Annals NY Acad. Sci. 799: 19-25 (1996).
Wang, et al. (1994) "Changing Enxymic Reaction Mechanisms by Mutagenesis: Conversion of a Retaining Glucosidase to an Inverting Enzyme", J. Am. Chem. Soc. 116:11594-11595.
Svensson, (1988) FEBS Letters 230:72-76.
Nagashima, et al. (1992) Biosci. Biotech. Biochem. 56:207-210.

ART-UNIT: 162
PRIMARY-EXAMINER: Slobodyansky; Elizabeth
ATTY-AGENT-FIRM: Oppedahl & Larson LLP

ABSTRACT:

Mutant glycosidase enzymes are formed in which the normal nucleophilic amino acid within the active site has been changed to a non-nucleophilic amino acid. These enzymes cannot hydrolyze disaccharide products, but which can still form them. Using this enzyme, oligosaccharides are synthesized by preparing a mixture of an .alpha.-glycosyl fluoride and a glycoside acceptor molecule; enzymatically coupling the .alpha.-glycosyl fluoride to the glycoside acceptor molecule to form a glycosyl glycoside product using the mutant glycosidase enzyme; and recovering the glycosyl glycoside product. Particular enzymes include a mutant form of Agrobacterium .beta.-Glucosidase in which the normal glutamic acid residue at position 358 is replaced with an alanine residue.

2 Claims, 3 Drawing figures

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L2: Entry 1 of 1 File: USPT Feb 10, 1998

US-PAT-NO: 5716812
DOCUMENT-IDENTIFIER: US 5716812 A

TITLE: Methods and compositions for synthesis of oligosaccharides, and the products formed thereby

DATE-ISSUED: February 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CAX
MacKenzie; Lloyd	Vancouver			CAX
Wang; Qingping	Montreal			CAX

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The University of British Columbia	Vancouver			CAX	03

APPL-NO: 8/ 571175
DATE FILED: December 12, 1995

INT-CL: [6] C12P 19/44, C12P 19/12, C12N 15/00, C12N 9/24
US-CL-ISSUED: 435/74; 435/100, 435/172.1, 435/200, 435/201, 435/209, 536/4.1
US-CL-CURRENT: 435/74; 435/100, 435/200, 435/201, 435/209, 536/4.1
FIELD-OF-SEARCH: 435/74, 435/100, 435/172.1, 435/200, 435/201, 435/209, 536/4.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4918009	April 1990	Nilsson	435/73
☐	5246840	September 1993	Nilsson	435/101
☐	5372937	December 1994	Nilsson	435/74

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0226563	June 1987	EPX	
87/05936	October 1987	WOX	
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94/29477	December 1994	WOX	
95/18864	July 1995	WOX	
95/18232	July 1995	WOX	

OTHER PUBLICATIONS

Withers et al. (1992) Biochemistry 31, 9979-9985.
Svensson. (1988) FEBS Letters 230, 72-76..
Nagashima et al. (1992) Biosci. Biotech. Biochem. 56, 207-210.
Wang et al.(1994) J. Am. Chem Soc. 116, 11594-11595.
Withers et al., "Mechanistic Comsequences of Mutation of the Active Site Nucleophile GLU 358 in Agrobacterium .beta.-Glucosidase" Biochemistry 31: 9979-9985 (1992).
Trimbur et al., "A .beta.-Glucosidase from an Agrobacterium sp.: Structure and Biochemistry" in ACS Symposium Seris (1992) pp. 42-55.
Gebler et al., "Substrate-Induced Inactivation of a Crippled .beta.-Glucosidase Mutant: Identification of the labeled Amino Acid and Mutagenic Analysis of Its Role", Biochemistry 34: 14547-14553 (1995).
Wang et al., "Identifictaion of the Acid/Base catalyst in Agrobacterium faecalis .beta.-glucosidase by knietic analysis of mutants" Biochemistry 34: 14454-14562 (1995).
Wang et al., "Substrate-assisted Catalysis in Glycosidases" J. Amer. Chem. Soc. 117: 10137-1-138 (1995).
Witt et al., "6-Phospho-.beta.-galactosidases of Gram Positive and 6-phospho-.beta.-glucosidase B of Gram-Negative bacteria: comparison of structure and function by kinetic and immunological methods and mutageneisis of the lacG gene of Staphyloccous aureus" Protein Engineering 6: 913-920 (1993).
Nikolova et al., "Transglycosylation by Wild Type and Mutants of a .beta.-1,4-Glycosidase from Cellulomonas fimi (Cex) for synthesis of Oligosaccharides", Annals NY Acad. Sci. 799: 19-25 (1996).

ART-UNIT: 184
PRIMARY-EXAMINER: Wax; Robert A.
ASSISTANT-EXAMINER: Slobodyansky; Elizabeth
ATTY-AGENT-FIRM: Oppedahl & Larson

ABSTRACT:

Mutant glycosidase enzymes are formed in which the normal nucleophilic amino acid within the active site has been changed to a non-nucleophilic amino acid. These enzymes cannot hydrolyze disaccharide products, but can still form them. Using this enzyme, oligosaccharides are synthesized by preparing a mixture of an .alpha.-glycosyl fluoride and a glycoside acceptor molecule; enzymatically coupling the .alpha.-glycosyl fluoride to the glycoside acceptor molecule to form a glycosyl glycoside product using the mutant glycosidase enzyme; and recovering the glycosyl glycoside product. Particular enzymes include a mutant form of Agrobacterium .beta.-Glucosidase in which the normal glutamic acid residue at position 358 is replaced with an alanine residue.

17 Claims, 3 Drawing figures

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L2: Entry 1 of 1 File: USPT Feb 10, 1998

US-PAT-NO: 5716812
DOCUMENT-IDENTIFIER: US 5716812 A

TITLE: Methods and compositions for synthesis of oligosaccharides, and the products formed thereby

DATE-ISSUED: February 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CAX
MacKenzie; Lloyd	Vancouver			CAX
Wang; Qingping	Montreal			CAX

US-CL-CURRENT: 435/74; 435/100, 435/200, 435/201, 435/209, 536/4.1

CLAIMS:

We claim:
1. A method for synthesizing an oligosaccharide comprising the steps of:
(a) combining a glycosyl donor molecule and a glycoside acceptor molecule in a reaction mixture; and
(b) enzymatically coupling the donor molecule to the acceptor molecule using a mutant form of glycosidase enzyme to form the oligosaccharide, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of the wild-type enzyme, and said mutant enzyme being mutated to replace one of said amino acids having a carboxylic acid side chain with a different amino acid of comparable or smaller size, said different amino acid having a non-carboxylic acid side chain.
2. The method of claim 1, wherein the glycosidase enzyme is a stereochemistry retaining enzyme in which one of the carboxylic acid side chains in the active site functions as an acid/base catalyst and the other carboxylic acid side chain functions as a nucleophile, and wherein the amino acid having the nucleophilic carboxylic acid side chain is replaced in the mutant enzyme.
3. The method of claim 2, wherein the enzyme is a .beta.-glycosidase.
4. The method of claim 3, wherein the glycosyl donor molecule is an .alpha.-glycosyl fluoride.
5. The method of claim 4, wherein the .alpha.-glycosyl fluoride is an .alpha.-glucosyl fluoride.
6. The method of claim 4, wherein the .alpha.-glycosyl fluoride is an .alpha.-galactosyl fluoride.
7. The method of claim 1, wherein the enzyme is a .beta.-glycosidase.
8. The method of claim 1, wherein the enzyme is a .beta.-glucosidase.
9. The method of claim 8, wherein the enzyme is Agrobacterium .beta.-glucosidase in which amino acid 358 has been changed from glutamic acid to an amino acid with a non-carboxylic acid side chain.
10. The method of claim 8, wherein the enzyme is Agrobacterium .beta.-glucosidase in which amino acid 358 has been changed from glutamic acid to alanine.
11. The method of claim 1, wherein the acceptor molecule is an aryl-glycoside.
12. The method of claim 11, wherein the acceptor molecule is a nitrophenyl-glycoside.
13. The method of claim 1, wherein the glycosidase enzyme is a stereochemistry

inverting enzyme in which one of the carboxylic acid side chains in the active site functions as an acid catalyst and the other carboxylic acid side chain functions as a base catalyst, and wherein the amino acid having the carboxylic acid side chain which functions as a base catalyst is replaced in the mutant enzyme.

14. The method of claim 1, wherein the enzyme is a mutant form of human or porcine .alpha.-amylase in which amino acid 197 has been changed from aspartic acid to alanine.

15. The method of claim 1, wherein the enzyme is a mutant form of human or porcine .alpha.-amylase in which amino acid 197 has been changed from aspartic acid to an amino acid with a non-carboxylic acid side chain.

16. The method of claim 1, wherein the enzyme is a mutant form of yeast .alpha.-glucosidase in which amino acid 216 has been changed from aspartic acid to alanine.

17. The method of claim 1, wherein the enzyme is a mutant form of yeast .alpha.-glucosidase in which amino acid 216 has been changed from aspartic acid to a non-carboxylic acid amino acid.